Published online November 21, 2006

Association of Root-Knot Nematode Resistance Genes with Simple Sequence Repeat Markers on Two Chromosomes in Cotton

Pavathi Ynturi, Johnie N. Jenkins,* Jack C. McCarty, Jr., Osman A. Gutierrez, and Sukumar Saha

ABSTRACT

Breeding for root-knot nematode (RKN) [*Meloidogyne incognita* (Kofoid & White) Chitwood] resistance in cotton (*Gossypium hirsutum* L.) is hindered by intensive screening procedures. Identification of DNA markers associated with RKN resistance would provide tools for marker-assisted selection (MAS). The objective of this study was to identify DNA markers associated with RKN resistance and associate these with chromosomes. Parents and an F_2 population from a cross of resistant near isoline (RNIL) × susceptible near isoline (SNIL) were grown in a greenhouse, inoculated with RKN eggs, and scored for gall index, followed by genotyping with simple sequence repeats (SSRs). The source of the resistance was from the Auburn 634 line. Genotype analysis was conducted on 86 F_2 plants with nine polymorphic SSR markers. Additive dominance model analysis showed that Brookhaven National Laboratory (BNL) SSR markers BNL 3661, 3644, 3545, and 1231 accounted for 21, 19, 12, and 11% of the variation in gall index, respectively. BNL 3661 and 1231 together accounted for 31% of the variation in gall index. BNL 3661 had significant additive and dominant genetic effects of 0.61 and 0.50, respectively. BNL 1231 had significant additive genetic effects of 0.51 and no dominant effects. BNL 3661, 3544, and 3645 were linked and these markers were located on the short arm of chromosome 14. BNL 1231 is located on the long arm of chromosome 11. The association of two different chromosomes with RKN resistance suggests at least two genes are involved in RKN gall score in the cross studied.

THE ROOT-KNOT NEMATODE is a serious pest of cotton. The nematode produces giant cells that act as metabolic sinks, reducing the ability of the root system to provide nutrients and water to the shoot. Conventional breeding programs for developing RKN resistance are time-consuming, labor-intensive, and may require both greenhouse and field evaluations. Identity of DNA markers closely associated with RKN resistant gene(s) will expedite the breeding process by allowing marker-assisted selection (MAS).

McPherson (1993) and McPherson et al. (2004) reported that a minimum of two genes control the high level of RKN resistance in M-315 RNR and one gene in moderately resistant M-78 RNR. Jenkins et al. (1995) data on postpenetration development of RKN in M-78 RNR and M-315 RNR also support the two-gene model. McPherson et al. (1995) reported that two major genes, one dominant and one additive gene controlled RKN resistance in Auburn 623 RNR-derived lines and named the dominant genes Mi_1 and the additive gene Mi_2. Bezawada et al. (2003) reported in their study that a single recessive gene controlled the RKN resistance trait in moderately resistant Clevewilt 6-1. They also reported weak association with BNL 1421; however, this marker showed distorted segregation which they indicated may have caused a false linkage. Wang et al. (2006a) reported a single recessive gene in linkage group A03 was responsible for the resistance in Acala NemX. They further reported SSR marker CIR 316a to be 2.6 CM away from this recessive gene they call rkn1 and SSR marker BNL 1231 was 18.4 units away. Zhou (1999) and Zhou et al. (1999) reported that a single recessive gene controlled the resistance in moderately resistant commercial cotton cultivars, Acala NemX and Stoneville LA 887, and two major genes in the resistant cotton line M-240 RNR (Shepherd et al., 1989, 1996). The later was derived from the Auburn 634 source (Shepherd, 1982, 1983). Turcotte et al. (1963) reported that two homozygous recessive genes conditioned the resistance in the F_2 population in a cross of *G. barbadense* L.

The objective of this research was to identify SSR markers associated with RKN genes and to assign these markers to specific chromosomes.

MATERIALS AND METHODS

A susceptible near isogenic line (SNIL), a resistant near isogenic line (RNIL), and an F_2 population of 86 plants were used in this study. The near isogenic lines were developed in our research program by crossing a highly resistant Auburn 634 RNR-derived line with susceptible cultivar Stoneville 213 (ST213) and backcrossing four times to ST213 while selecting resistant and susceptible sister lines.

We used the modified methods of Shepherd (1979) to screen for RKN resistance in the greenhouse. Roots from RKN, race 3, infected tomato, and cotton plants were placed in 1.05% NaOCl solution and placed on a mechanical shaker for 3 min following the method of Hussey and Barker (1973) to obtain inoculum.

Individual plants of parental lines (M8 susceptible check, M-315 RNR resistant check) and F_2 were grown in 8.9 × 7.6 cm (diameter × depth) plastic pots placed in equidistant holes of 6-cm depth in the greenhouse beds. The pots and greenhouse beds were filled with screened, methyl bromide-fumigated Wickham sandy loam soil (fine-loamy, mixed, semiactive, thermic Typic Hapludults). Those pots were inoculated with approximately 10 000 RKN eggs after planting a seed. The bed was covered for 7 d with sequential layers of brown paper and

P. Ynturi, Dep. of Plant and Soil Sciences, Mississippi State Univ., Mississippi State, MS, 39762; J.N. Jenkins, J.C. McCarty, Jr., O.A. Gutierrez, and S. Saha, USDA-ARS, Crop Science Research Lab., Mississippi State, MS 39762. Mention of trade names or commercial products in this manuscript does not imply recommendations or endorsement by the USDA. Joint Contribution of USDA, ARS, and Mississippi State University. Journal paper No.J-10952 of Mississippi Agricultural and Forestry Experiment Station. Received 15 May 2006. *Corresponding author (jnjenkins@ars.usda.gov).

Published in Crop Sci. 46:2670–2674 (2006).
Crop Breeding & Genetics
doi:10.2135/cropsci2006.05.0319

677 S. Segoe Rd., Madison, WI 53711 USA

Abbreviations: BNL, Brookhaven National Laboratory; LOD, log likelihood ratio; MAS, marker-assisted selection; RKN, root-knot nematode; RNIL, resistant near isogenic line; SNIL, susceptible near isogenic line; ST213, Stoneville 213; SSR, simple sequence repeats.

black plastic to allow for hatching and dispersal of the juveniles into the soil and germination of seed. Eighty-six F_2 plants from RNIL × SNIL, 30 of each of the parental lines, 30 M8 (susceptible check), and 30 M-315 RNR (resistant check) plants were grown and scored for gall index. Six weeks after emergence, the plants were excised approximately 5 cm from the soil line and the soil was gently washed away from roots with running water. The washed roots were held in pots filled with water for root gall scoring. The root galling was rated on each plant, individually by three persons using a 1 to 5 index (Shepherd, 1983), where gall indices 1 = plants with no galls or very few galls, 2 = plants with a few small-sized galls, 3 = plants with a moderate number of galls, mostly medium sized, 4 = plants with many large-sized galls, 5 = all most all the roots in the plant covered with large galls. Data used for analysis are mean gall index of plants rated by three people. Thus, mean index is recorded with one decimal place.

Leaf samples were collected from individual plants, placed in liquid nitrogen, freeze-dried, and ground before storing in a freezer at −50°C (Saha et al., 1997). DNA from 150 mg stored leaf samples of five individual plants of each of the parental lines and 86 individual F_2 plants of the RNIL × SNIL cross was extracted using a DNeasy Plant Mini Kit (Qiagen, Santa Clarita, CA) following the manufacturer's protocol.

Fluorescent-labeled 5′ SSR primers and unlabeled 3′ SSR primer pairs used in this study were purchased from Sigma Genosys (The Woodlands, Texas) and PE Applied Biosystems, (Foster City, CA). The fluorescent-labeled 5′ SSR primers are labeled with 6-FAM (6-carboxyfluorescein), HEX (4,7,2′,4′,5,7-hexachloro-carboxyfluorescein) and NED (7′,8′-benzo-5-fluoro-2′,4,7,-trichloro-5-carboxyfluorescein).

Gene Amp PCR reagent kits (PE Applied Biosystems, Foster City, CA) were used to perform a polymerase chain reaction (PCR). The genomic DNA sample was amplified using SSR primers in 10 μL reactions containing 1× Gene Amp PCR Gold Buffer, 0.15 μ*M* SSR primers, 2.5 m*M* $MgCl_2$, 0.5 units Ampli Taq Gold DNA polymerase, 0.2 m*M* $dNTP_s$, and 20 ng of the template DNA sample. The PCR was conducted according to the method of Gutierrez et al. (2002).

The RNIL and SNIL parents were screened with 326 Brookhaven National Laboratory (BNL) SSR markers to identify polymorphic markers. The 15 identified polymorphic markers were used to screen 10 highly resistant (lowest gall index) and 10 highly susceptible (highest gall index) individual plants of the RNIL × SNIL F_2 population in a modified bulk segregate analysis to find markers likely to be associated with resistance or susceptibility. The nine potential associated markers were then used to screen the 86 individual F_2 plants.

An automated capillary electrophoresis system ABI 3100 genetic analyzer (PE Appl. Biosystems, Foster City, CA) with GeneMapper software version 3.5 (PerkinElmer, Norwalk, CT) was used to run and analyze the PCR-amplified DNA fragments following the overall method of Gutierrez et al. (2002). To eliminate the effect of comigration and instrument noise, an SSR marker was considered to be a valid data point only if peak height on the *Y*-axis was at least 100 fluorescent units and the peaks showed at least a 1 bp difference from the closest marker on the *X*-axis. Variations in the amplified products were compared with an internal DNA size standard labeled with the ROX dye. Computer-assisted analysis of the data was performed with GeneMapper Software v3.5.

Single-factor analyses of variance were conducted in SAS Proc GLM (SAS Institute, 2001) to estimate additive and dominant effects associated with each marker in the data set (Edwards et al., 1987). Markers that had significant individual effects were then considered for developing multiple-marker models in SAS Proc GLM. The marker with highest significance was entered into the multiple-marker model first, followed by the locus with second-highest significance, and so forth. At each step, the significance of each locus was retested based on Type III sums of squares. Any loci not significant at $P < 0.05$ in the multiple locus model were dropped from the model. This process continued until no additional markers could be added that remained significant in the multiple marker model, resulting in a final model. In addition, each significant marker locus was tested for epistatic interactions with all other significant marker loci using SAS Proc GLM (Holland, 1998).

Join Map 3.0 (Van Ooijen and Voorrips, 2001) software was used to calculate genetic linkage in the F_2 population. SSR peaks were scored as present (1) or absent (0) by the ABI GeneMapper v3.5 software and verified by visual inspection. Data coded (0) and (1) were transformed to A, B, and H genotype codes according to the presence of the resistant or susceptible parent fragment or the presence of both fragments, following the Join Map procedure. Linkage groups were obtained using a log likelihood ratio (LOD) score of 4 and a maximum recombination frequency of 0.45. To check marker segregation, Chi-square tests were used. For the codominant molecular markers, segregation was tested against an expected 1:2:1 frequency and dominant molecular marker segregation was tested against an expected 3:1 frequency.

Cytologically identified monosomic and monotelodisomic chromosome substitution lines (BC_0F_1), developed from a cross between aneuploid or monotelodisomic TM-1 (*G. hirsutum* L.) variants and euploid 3-79 (*G. barbadense L.*), were used to identify the chromosomal location of the SSR markers based on a deletion method (Saha and Stelly, 1994; Liu et al., 2000; Karaca et al., 2002). F_1 plants used were from the monotelodisomes for 1Lo, 1sh, 2Lo, 2sh, 3Lo, 3sh, 4Lo, 4sh, 5Lo, 6Lo, 6sh, 7Lo, 7sh, 9Lo, 10Lo, 10sh, 11Lo, 12Lo, 14Lo, 15Lo, 16Lo, 16sh, 17sh, 18Lo, 18sh, 20Lo, 20sh, 22Lo, 22sh, 25Lo, 26Lo, and 26sh and from monosomes H1, H2, H3, H4, H6, H7, H9, H10, H11, H12, H16, H17, H18, H20, H23, and H25.

The markers associated with RKN resistance gene loci were tested for polymorphisms between parents TM-1 and 3-79. These polymorphic markers were then screened across the F_1 cytogenetic stocks to identify their chromosomal association. When a cytogenetic stock showed the presence of both TM-1 and 3-79 alleles, it was considered that the marker allele was not located on the particular deficient chromosome or chromosome arm but if the plant exhibited a hemizygous pattern, i.e., TM-1 allele was missing, whereas 3-79 band was present, then it was considered that the marker was located on the missing chromosome or chromosome arm of the plant.

RESULTS AND DISCUSSION

The mean gall index ranged from 1.0 to 2.3 for M-315 RNR and the range in the M8 and ST213 susceptible populations was 2.7 to 5.0 with most plants scoring 4.0 or greater. The SNIL index ranged from 3.7 to 5 with most plants scoring 4.7 or 5.0. The RNIL index ranged from 1.0 to 3.7 with six plants scoring greater than 2.3. The frequency distribution of the gall index of RNIL × SNIL F_2 plants is shown in Table 1.

McPherson et al. (2004), using lines from the Auburn 634 source, suggested a two-gene model and the designations of Mi_1 and Mi_2 for the two genes. Wang et al. (2006a) reported a single recessive gene (rkn_1) responsible for the resistance in NemX. They further identified a marker Cir316a that was only 2 centimorgans away from this recessive gene and reported it to be in linkage group A03.

Table 1. Segregation of root-knot nematode gall index among 86 F_2 plants from a cross of RNIL × SNIL.

Parameter	RNIL × SNIL Cross														
Gall index	1.0	1.3	1.7	2.0	2.3	2.5	2.7	3.0	3.3	3.5	3.7	4.0	4.3	4.7	5.0
No. plants	5	3	1	9	6	1	3	12	5	0	13	6	11	4	7

We found 14 BNL polymorphic SSR markers between the RNIL and SNIL parents. We did not expect a large number of polymorphic markers since these two lines are near isolines which differ for resistance genes. These14 polymorphic markers were used to individually genotype 10 highly resistant and 10 highly susceptible F_2 individual plants. Nine BNL markers appeared to be linked with resistance (Table 2). These nine potential polymorphic markers were used to screen 86 individual F_2 plants. Seven markers segregated as codominant and two (BNL 3977 and 3537) as dominant markers and all markers segregated normally, except BNL 3977 where there were more plants than expected with the marker allele from the RNIL parent.

Marker data showed that BNL 3661, BNL 3545, BNL 3644, and BNL 1231 were significantly associated with the RKN gall index, whereas the other five markers were not. Twenty one percent of the gall index variation was explained by BNL 3661, 19% by BNL 3644, 12% by BNL 3545, and 11% by BNL 1231 (Table 3). BNL 3661 and BNL 3644 explained 26%; BNL 3661 and BNL 1231 explained 31% (Table 4). BNL 3661 exhibited significant additive and dominant genetic effects, whereas BNL 1231 had only significant additive genetic effects. When the additive dominance model contained BNL 3661 and BNL 1231, additive and dominant genetic effects for BNL 3661 and additive effects for BNL 1231 were indicated (Table 4) and thus a two-gene model was indicated. No epistasis was found. Since only 31 to 37% of the variation in gall index could be explained by the markers on chromosome 11 and 14, the possibility exists that other genes may be involved in resistance that could not be detected because of lack of polymorphisms.

A linkage map was constructed using Join Map 3.0 software. The one linkage group found consisted of BNL 3661, BNL 3644, and BNL 3545 (Fig. 1) with a LOD score of 4. These markers were also found associated with RKN resistance in the regression analysis. Ynturi (2005) reported that chromosomes 14 and 20 (thus at least two genes) were involved and that BNL 1231 was on chromosome 20; however, our current data place BNL 1231 on chromosome 11. The genetic distance between BNL 3661 and BNL 3644 was 7 cM and between BNL 3644 and BNL 3545 was 4 cM. BNL 1231, although associated with resistance, was not associated with this linkage group, further suggesting that two genes are involved in resistance.

The four codominant SSR markers associated with RKN resistance were polymorphic between the euploid parents TM-1 and 3-79, thus they could be assigned to chromosomes. TM-1 and 3-79 are the parents of the cytogenetic F_1 stocks used to assign markers to chromosomes. When screened against monosomic and monotelodisomic F_1 cytogenetic stocks, BNL 3661, BNL 3644, and BNL 3645 were missing on Te14Lo (short arm 14 missing in the plant). This indicates that these marker alleles are located on the short arm of chromosome 14. We will now screen additional chromosome 14 markers to further fine map the location of this gene. BNL 1231 was reported by Wang et al. (2006b) to be in linkage group A03. Gutierrez (unpublished data, 2005) showed that BNL 1231 was present on Te11Lo but missing on the H11 when the entire TM-1 chromosome 11 was missing, thus placing BNL marker 3661 on the long arm of chromosome 11. Wang et al. (2006b) using fluorescence in situ hybridization mapping showed that A03 is chromosome 11. This indicates that two chromosomes, the short arm of chromosome 14 and the long arm of chromosome 11, contain markers associated with RKN resistance. This further confirms that two genes for resistance are probably correct. It is interesting that the recessive gene in our line and the recessive gene in NemX are in the same linkage group. This may indicate a resistance gene cluster or they may not be closely linked.

Our data show that the gene on the short arm of chromosome 14 linked to BNL 3661 is a gene with dominant and additive genetic effects, whereas the gene linked with BNL 1231 on chromosome 11 only has additive genetic effects. We could not test for association of BNL 1421 that Bezawada et al. (2003) suggested as possibly linked with RKN resistance because it was not poly-

Table 2. Polymorphic SSR markers and allele size in the RNIL and SNIL parents.

Primer	RNIL Allele size: base pair	SNIL Allele size: base pair	Type
BNL 598	123	125	codominant
BNL 1231	195	189	codominant
BNL 1673	168	195	codominant
BNL 3537	187	–	dominant
BNL 3545	117	137	codominant
BNL 3644	192	188	codominant
BNL 3661	185	195	codominant
BNL 3875	135	133	codominant
BNL 3977†	124	–	dominant

† Distorted segregation.

Table 3. Genetic effects and coefficients of determination associated with single markers and root-knot nematode gall index using the additive dominance model with 86 F_2 plants.

Marker	R^2	Gall index			Additive effect	Dominant effect
		Mean A†	Mean B‡	Mean H§		
BNL 3661	0.21**	2.9	4.1	2.9	0.56**	−0.61**
BNL 3644	0.19**	2.6	3.9	3.1	0.69**	−0.22
BNL 3545	0.12**	2.5	3.7	3.2	0.60**	0.0
BNL 1231	0.11**	2.8	3.8	3.2	0.52**	−0.11
BNL 598	0.00	3.4	3.1	3.2	−0.14	0.02
BNL 3537	0.00	3.2	3.3	–	0.00	–
BNL 1673	0.02	3.4	2.9	3.3	−0.27	0.15
BNL 3977	0.00	3.2	3.3	–	0.08	–
BNL 3875	0.00	3.3	3.2	3.2	−0.03	−0.07

** Significant at 0.01 level.
† A is mean gall index of plants with only marker allele of resistant parent.
‡ B is mean gall index of plants with only marker allele of susceptible parent.
§ H is mean gall index of plants with one marker allele from each parent.

Table 4. Genetic effects and coefficients of determination associated with multiple markers and root-knot nematode gall index using the additive dominance model.

Markers	R^2	Additive effect	Dominant effect
Additive dominance model			
BNL 3661 + 3644	**0.26****	**–**	**–**
BNL 3661	**–**	**0.30**	**−0.99****
BNL 3644	**–**	**0.36**	**0.56**
BNL 3661 + 1231	**0.31****	**–**	**–**
BNL 3661	**–**	**0.61****	**0.50***
BNL 1231	**–**	**0.51****	**0.10**
BNL 3661 +3644 + 3545	**0.28****	**–**	**–**
BNL 3661	**–**	**0.28**	**−0.95****
BNL 3644	**–**	**0.59**	**0.08**
BNL 3545	**–**	**0.23**	**0.52**
BNL 3661 + 3644 + 3545 + 1231	**0.37****	**–**	**–**
BNL 3661	**–**	**0.24**	**0.66**
BNL 3644	**–**	**0.64**	**0.18**
BNL 3545	**–**	**0.20**	**0.57**
BNL 1231	**–**	**0.50****	**0.00**

*** Significant at 0.05 levels.**
**** Significant at 0.01 levels.**

morphic in the RNIL and SNIL parents. However, our data would suggest that linkage with BNL 1421 is probably spurious since this marker is located on chromosome 16 and Bezawada et al. (2003) indicated that the linkage might be spurious because of the abnormal segregation of this marker in their population. McPherson et al. (2004), our current data, Zhou (1999), and Zhou et al. (1999) report a two-gene model for resistance in the M-315, RNIL, and M-240 sources, respectively. Considering our current data with the published data, there are genes on two chromosomes involved in the Auburn 634 source of RKN resistance and we have identified SSR markers associated with these genes. This information should be very useful in commercial breeding programs for resistance to RKN.

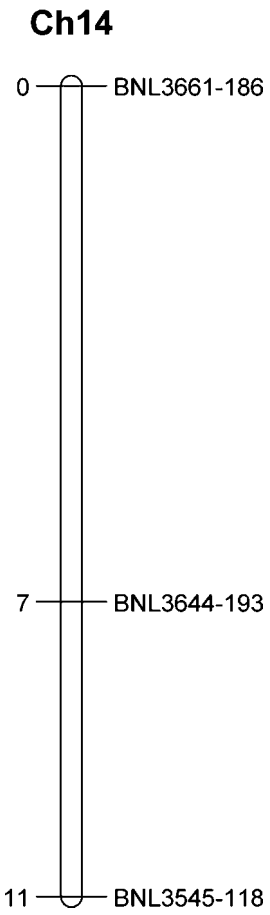

Fig. 1. Chromosome 14 linkage group associated with RKN resistance.

ACKNOWLEDGMENTS

The authors thank Dr. David Stelly of Texas A & M University for supplying the cytogenetic stocks utilized in assigning the markers to chromosomes.

REFERENCES

Bezawada, C., S. Saha, J.N. Jenkins, R.G. Creech, and J.C. McCarty. 2003. [Online] SSR Marker(s) associated with root-knot nematode resistance gene(s) in cotton. J. Cotton Sci. 7:179–184.

Edwards, M.D., C.W. Stuber, and J.F. Wendel. 1987. Molecular-marker-facilitated investigations of quantitative-trait loci in maize: I. Numbers, genomic distribution, and types of gene action. Genetics 116:113–125.

Gutierrez, O.A., S. Basu, S. Saha, J.N. Jenkins, D.B. Shoemaker, C. Cheatham, and J.C. McCarty, Jr. 2002. Genetic distance among selected cotton genotypes and its relationship with F_2 performance. Crop Sci. 42:1841–1847.

Holland, J. 1998. EPISTACY: A SAS program for detecting two-locus epistatic interactions using genetic marker information. J. Hered. 89:374–375.

Hussey, R.S., and K.R. Barker. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., include a new technique. Plant Dis. Rep. 57:1025–1028.

Jenkins, J.N., R.G. Creech, B. Tang, G.W. Lawrence, and J.C. McCarty. 1995. Cotton resistance to root-knot nematodes: II. Post-penetration development. Crop Sci. 35:369–373.

Karaca, M., S. Saha, J.N. Jenkins, A. Zipf, R. Kohel, and D.M. Stelly. 2002. Simple sequence markers (SSR) linked to the Ligon Lintless (Li1) mutant in cotton. J. Hered. 93(3):221–224.

Liu, S., S. Saha, D.M. Stelly, B. Burr, and R.G. Cantrell. 2000. Chromosomal assignment of microsatellite loci in cotton. J. Hered. 91: 326–332.

McPherson, G.R. 1993. Inheritance of root-knot nematode resistance in cotton as determined by combining ability, generation mean, and Mendelian analysis. Ph.D. diss. Mississippi State Univ., Starkville, MS (DAI-B54/08).

McPherson, G.R., J.N. Jenkins, J.C. McCarty, Jr., and C.E. Watson. 1995. Combining ability analysis of root-knot nematode resistance in cotton. Crop Sci. 35:373–375.

McPherson, M.G., J.N. Jenkins, C.E. Watson, and J.C. McCarty, Jr. 2004. Inheritance of root-knot nematode resistance in M315 RNR and M78-RNR cotton. J. Cotton Sci. 8:154–161.

Saha, S., F.E. Callahan, D.A. Dollar, and J.B. Creech. 1997. Effect of lyophilization of cotton tissue on quality of extractable DNA, RNA, and protein. J. Cotton Sci. 1:11–14.

Saha, S., and D.M. Stelly. 1994. Chromosome location of phosphoglucomutase 7 locus in Gossypium hirsutum. J. Hered. 85:35–39.

SAS Institute. 2001. SAS software version 8.0. SAS Institute, Cary, NC.

Shepherd, R.L. 1979. A quantitative technique for evaluating cotton for root-knot nematode resistance. Phytopathology 69:427–430.

Shepherd, R.L. 1982. Registration of three germplasm lines of cotton (Reg. Nos. GP 164–166). Crop Sci. 22:692.

Shepherd, R.L. 1983. New sources of resistance to root-knot nematodes among primitive cotton. Crop Sci. 23:999–1002.

Shepherd, R.L., J.C. McCarty, Jr., J.N. Jenkins, and W.L. Parrott. 1996. Registration of nine germplasm lines resistant to root-knot nematode. Crop Sci. 36:820.

Shepherd, R.L., W.L. Parrott, J.C. McCarty, Jr., and J.N. Jenkins. 1989. Notice of release of nine root-knot nematode resistant germplasm lines of upland cotton *Gossypium hirsutum* L. USDA-ARS and Mississippi Agric. and Forestry Exp. Station Memo. USDA, Washington, DC.

Turcotte, E.L., W.R. Harold, J.H. O'Bannon, and C.V. Feaster. 1963. Evaluation of cotton root-knot nematode resistance of a strain of G. barbadense Var. darwinni. p. 36–44. *In* Cotton Improvement Conf. (15th), Dallas, TX. 8–9 Jan. Natl. Cotton Council Am., Memphis, TN.

Van Ooijen, J.W., and R.E. Voorrips. 2001. JoinMap3.0 Software for the calculation of genetic linkage maps. Plant Research International, Wageningen, the Netherlands.

Wang, C., M. Ulloa, and P.A. Roberts. 2006a. Identification and mapping of microsatellite markers linked to a root-knot nematode resistance gene (rkn1) in Acala NemX cotton (G*ossypium hirsutum* L). Theor. Appl. Genet. 112:770–777.

Wang, K., X. Song, Z. Han, W. Guo, J. Sun, J. Pan, R.J. Kohel, and T. Zhang. 2006b. Complete assignment of the chromosomes of Gossypium hirsutum L. by translocation and fluorescence in situ hybridization mapping. Theor. Appl. Genet. 113:73–80.

Ynturi, P. 2005. Phenotypic and genotypic study of root-knot nematode resistance in near isogenic lines of cotton. M.S. thesis. Mississippi State University, Starkville, MS.

Zhou, E. 1999. Resistance to *Meloidogyne incognita* in cotton genotypes. Ph.D. diss. (Diss. Abstr. Int. 61–01B:24). Texas A&M University, College Stn., TX.

Zhou, E., J.L. Starr, and C.W. Smith. 1999. Inheritance of resistance to *Meloidogyne incognita* in the cotton cultivar Acala NemX. J. Nematol. 31:584–585.